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<b>(54) Title:</b> USE OF CAMPTOTHECIN OR DERIVATIVES THEREOF FOR THE MANUFACTURE OF A MEDICAMENT FOR THE TREATMENT OF VIRAL DISEASES		
<b>(57) Abstract</b>  The present invention provides methods of inhibiting viral replication by preparing an effective dosage of a camptothecin compound and introducing the effective dosage to the virus. Retrovirus, DNA virus, and topoisomerase I containing virus replication is inhibited. The camptothecin compound may be 9-amino-20(S)-camptothecin, 9-nitro-20(S)-camptothecin, 20-(S)-camptothecin, or any other active camptothecin compound, active semisynthetic camptothecin analogue, or active synthetic camptothecin analogue. One or more antiviral drugs may be combined with one or more of the aforementioned camptothecin compounds. A method of treating viral diseases in mammals comprising preparing an effective dosage of a camptothecin compound selected from the group consisting essentially of camptothecin, active semisynthetic camptothecin analogues, active synthetic camptothecin analogues, and combinations thereof. The dosage is administered to the infected mammal. Pharmaceutical formulations are also provided.		

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USE OF CAMPTOTHECIN OR DERIVATIVES THEREOF FOR THE MANUFACTURE OF A MEDICAMENT  
FOR THE TREATMENT OF VITAL DISEASES

FIELD OF THE INVENTION

The invention relates to a method of inhibiting viral replication to treat and prevent disease. Particularly, diseases produced in mammals by retroviruses, DNA viruses, DNA-mediated RNA-containing viruses and viruses containing viral topoisomerase I may be prevented or treated according to this invention.

BACKGROUND OF THE INVENTION

It was estimated in 1993 that human immunodeficiency virus (HIV), a lentiretrovirus, had infected approximately one million people in the United States. In approximately 280,000 of those infected, the infection had progressed to acquired immunodeficiency disease (AIDS), resulting in nearly two-thirds of them dying as a result of the infection. Breo, D.A., JAMA, 269 (22): 2898 (1993). It has also been estimated that thirteen million people worldwide are infected with the HIV virus, and that several million of those infected have progressed to AIDS. It has been anticipated that by the year 2000, fifty to one hundred million people will have been infected by HIV. The currently available drugs for treating HIV, such as Zidovudin (3'-azido-2', 3' did oxythymidin or AZT), Didanosine (2', 3' did oxyinosine or DDI), and Hividzalcitabine (2', 3' dideoxycytidine or DDC), off r

only transient benefit and are relatively toxic to the patient. Bro, D.A., JAMA, 269 (22): 2898 (1993).

Many other diseases are the result of viral infection. Although such diseases may be less devastating than AIDS, they frequently infect larger segments of the population. For example, according to estimates, there are more than two hundred million hepatitis B, a hepadnavirus, carriers in the world. Dienstag, J.L., et al., in *Harrison's Principles of Internal Medicine* (12th Ed.), McGraw-Hill, New York, p. 1326, (1991). In addition to the overwhelming health problems produced by the AIDS and hepatitis B viruses, the many diseases produced, for example, by herpesviruses, papovaviruses, and parvoviruses, pose major health concerns to humans and animals.

In 1966, crude extracts of the tree *Camptotheca acuminata* (Nyssaceae) were found to be active against lymphoid leukemia L-1210 in mice by the antitumor screening program of the Cancer Chemotherapy National Science Center (CONSC) National Center Institute. Abbott, B.J. et al., *Cancer Res.* 26 (No. 2, pt 2), 34 (1966). The alkaloid camptothecin was isolated along with 10-methoxycamptothecin and the structures were characterized at the Research Triangle Institute, Durham, N.C. Wall, M.E., et al., 4th *Internatl. Symp. Nat. Prod., Internatl Union Pure and Appl. Chem., Stockholm*, 103 (1966); Wall, M.E., et al., *J. Am. Chem. Soc.* 88 (16) 3888 (1966). Both camptothecin and 10-methoxycamptothecin have been isolated from the southern India and Ceylon shrub, *Ophirrhiza Mungos*, Linn (Rubiaceae) and from *Mappia foetida* (Olinaceae). Tafur, S., et al., *Lloydia*, 39 no. 4, 261 (1976); Agarval, J.L., et al., *Indian J. Chem.*, 11, 969 (1973).

Camptothecin was later found to inhibit both DNA and RNA synthesis in mammalian cells and to cause partially reversible fragmentation of DNA. S.B. Horwitz, camptothecin in J.W. Corcoran and F.E. Hahn (eds), *Antibiotics III*, New York, Springer and Verlag, 48 (1975);

Hsiang, Y-H., et al., *Cancer Res.* 49, 4385 (1989). The mode of action of camptothecin was reported as inhibition of the mammalian cellular enzyme DNA topoisomerase I. Hsiang, Y-H., et al., *J. Biol. Chem.* 260, 14873 (1985).

5       Topoisomerase I produces a transient break in a single strand of DNA (D'Arpa, P., et al., *Biochem, Biophys, Acta* 989, 163 (1989)), producing a covalent enzyme intermediate labelled the cleavable complex (Wang, J.C., *Am. Rev. Biochem.*, 54, 665 (1985)). Camptothecin blocks the  
10    rejoining step of the breakage-reunion reaction of topoisomerase I. Studies demonstrate that camptothecin and its active analogues preferentially stabilize a subset of adducts that contain DNA topoisomerase I covalently bound to the 3' phosphate group of thymidine to yield a free 5'-  
15    OH group on a guanosine residue Jaxel, C., et al., *Nucleic Acids Res.*, 16, 1157 (1980); Jaxel, C., et al., *J. Biol. Chem.* 226, 20418 (1981). After topoisomerase I inhibition, cell death results from stabilizing topoisomerase I-DNA adducts, some of which are converted to irreversible  
20    cytotoxic double strand breaks through an interaction with the replication apparatus. Zang, H., et al., *Cancer Cells*, 2, 23, (1990).

Semi-synthetic and fully synthetic analogues of camptothecin have been prepared and studied for their  
25    anticancer activity in human cancer xenografts in immunodeficient mice Hsiang, Y-H, et. al., *Cancer Res.*, 49, 4385 (1989); Giovanella, B.C., et al., *Cancer Res.*, 51, 3052 (1991). Anticancer activity has varied from extremely active in some analogues to totally inactive in others.  
30    Among the highly active analogues are 9-aminocamptothecin (labile in solution and may oxidize to inert), 9-nitrocamptothecin (solution stable) and 10, 11-methylene-dioxycamptothecin. CPT-11 (7-ethyl-10 [4-(1-piperidino) - 1- piperidino] carbonlyoxycamptothecin) and Topotecan (9-  
35    dimethyl-aminomethyl-10-hydroxycamptothecin) are water soluble analogues of 1 ss antitumor activity.

- Sodium camptothecin was shown to induce in vitro inhibition of DNA replication and viral morphogenesis of Adenovirus type 2 and Vaccinia virus in infected cells. Horwitz, M.S., et al., *Biochem. and Biophysics Res. Commun.*, 45, 723 (1971); Horwitz M.S., et al., *Virology* 48, 690 (1972). Camptothecin has also been shown to completely inhibit the in vitro synthesis of herpes simplex virus in infected cells. Becker Y., et al., *Isr. J. Med. Sci.*, 9, 1578 (1976); Tafur, S., et al., *Lloydia*, 39, 261 (1976).
- 10 Prevention and treatment of lymphoma from Maloney murine leukemia virus in newborn BALB/c mice and prevention and treatment of erythroleukemia from Friend spleen focus-forming virus in adult NFS mice by intraperitoneally injected camptothecin 5 mg/kg has also been reported.
- 15 Priel, E., et al., *J. of Virol.*, 67, 3624 (1993).

- Viral DNA topoisomerase I was reported present in HIV-1, equine infectious anemia virus and Maloney murine leukemia virus. Priel, E., et al., *EMBO J.* 9, 4167 (1990). About .05 $\mu$ M or 18.1 ng/ml of camptothecin reportedly
- 20 inhibited HIV-1 replication acutely and chronically in H-9 cells grown in tissue culture. Priel, E., et al., *AIDS Res. and Human Retroviruses*, 7 (1), 65 (1991). In the report re: IND NUMBER 39,272 submitted July 1, 1993 and accepted August 1, 1993, by the U.S. Food and Drug
- 25 Administration from The Stehlin Foundation for Cancer Research entitled "Report of phase-I clinical trial with oral administration of 20-(S)-camptothecin" which was conducted in cancer patients, oral doses of camptothecin produced plasma concentrations of camptothecin in patients
- 30 ranging from 8.4  $\mu$ g/ml to 7.89  $\mu$ g/ml. Six patients in the study had taken oral 20-(S)-camptothecin in therapeutic doses for over 14 months.

- Approximately 57% to 75.67 % of orally administered 9-nitro-(S)-camptothecin has been shown to convert
- 35 intracellularly to 9-amino-(S)-camptothecin. High therapeutic blood levels of 9-amino-(S)-camptothecin are

observed following oral administration of 9-nitro-(S)-camptothecin in mice, dogs, and humans, with minimal or no side effects. Following a single oral dose of 0.1 mg/kg (1 mg/m<sup>2</sup>) of 9-nitro-(S)-camptothecin, the human plasma concentration reached a maximum of 483 nanograms/milliliter (ng/ml) at 3.4 hours. The area under the curve (AUC) was 208 ng·hr/ml with a half life of 2.5 hours. As conversion of 9-nitro-(S)-camptothecin to 9-amino-(S)-camptothecin occurred, the maximum calculated concentration of 9-amino-(S)-camptothecin was 14 ng/ml at 10.3 hours. The AUC was 311 ng·hr/ml with a half life of 7.1 hours. No toxicity was observed. Hinz, H.R., et al., *Cancer Res.* 54: 3096-3100 (1994). A dose of 0.1 mg/kg produced no discernable side effects in a volunteer taking the compound 5 days and skipping 2 days per week for a total of 3 weeks. A patient taking 0.1 mg/kg daily for over 6 weeks showed no ill effects.

The replication of HIV-1 requires that the single stranded RNA molecule serve as a template for reverse transcriptase to generate a double stranded DNA copy of the genome. The DNA copy is integrated into the cell nucleus through viral integrase and becomes a provirus in the host cell genome. The provirus is replicated along with the cellular DNA. Transcription of the provirus is governed by the viral long terminal repeat. Topotecan, a soluble camptothecin analogue, at a concentration of 0.3  $\mu$ M is a selective inhibitor of HTLV-111<sub>B</sub> long terminal repeat-directed gene expression at noncytotoxic concentrations in tissue culture. Topotecan inhibits p 24 antigen production in both acute and chronic infection of HTLV-111<sub>B</sub> in human peripheral blood mononuclear cells in tissue culture. Li, C.J., et al., *Proc. Natl. Acad. of Sci., USA*, 90, 1839 (1993).

The method and use of camptothecin, active semisynthetic analogues and synthetic analogues can be used as a treatment and a preventative for virus, including human

immunodeficiency virus (HIV), and other diseases produced by retroviruses, DNA containing viruses, DNA-mediated RNA-containing viruses, and viruses containing viral topoisomerase I. Effective camptothecin compounds include 9 aminocamptothecin and 9 nitrocamptothecin. Camptothecin and its analogues can be used with other antiviral drugs in combination for viral disease treatment and prevention.

#### SUMMARY OF THE INVENTION

A method of inhibiting viral replication is achieved by preparing an effective dosage of a camptothecin compound selected from the group consisting essentially of camptothecin, active semisynthetic camptothecin analogues, active synthetic camptothecin analogues, and combinations thereof. The effective dosage is then introduced to a virus. The virus may be selected from the group of viruses consisting essentially of retroviruses, DNA viruses, DNA-mediated RNA-containing viruses, and viruses containing viral topoisomerase I. The preferred camptothecin compound is 9-nitro-20(S)-camptothecin. In alternate embodiments, 9-nitro-20(RS)-camptothecin, 9-amino-20(S)-camptothecin, 9-amino-20(RS)-camptothecin, 20-(S)-camptothecin, 20-(RS)-camptothecin and other analogues may be used. An antiretroviral compound selected from the group consisting essentially of HIV long terminal repeat inhibiting compounds, reverse transcriptase inhibitors, protease inhibitors, TAT inhibitors, rev protein inhibitors, and responsive element (rre) inhibitors, and combinations thereof may be combined with the effective dosage of camptothecin compound. Immunoadjuvant drugs, cytokine agonists, cytokine inhibitors, drugs that prevent viral attachment to cell surface receptors, and combinations thereof may also be combined with the camptothecin compounds. The effective dosage may be introduced to the virus one or more times per day on one or more times per month for a selected period of time.



A method of treating viral diseases in mammals is achieved by preparing an effective dosage of a camptothecin compound selected from the group consisting essentially of camptothecin, active semisynthetic camptothecin analogues, 5 active synthetic camptothecin analogues, and combinations thereof and administering the effective dosage to a virus infected mammal. The camptothecin compound may also be administered to prevent the viral disease from occurring in the virus infected mammal. The preferred camptothecin 10 compound is 9-nitro-20(S)-camptothecin. In alternate embodiments, 9-nitro-20(RS)-camptothecin, 9-amino-20(S)-camptothecin, 9-amino-20(RS)-camptothecin, 20-(S)-camptothecin, 20-(RS)-camptothecin and others may be used. An antiretroviral compound selected from the group 15 consisting essentially of HIV long terminal repeat inhibiting compounds, reverse transcriptase inhibitors, protease inhibitors, TAT inhibitors, rev protein inhibitors, rre inhibitors, and combinations thereof may be combined with the effective dosage of camptothecin 20 compound. Immunoadjuvant drugs, cytokine agonists, cytokine inhibitors, drugs that prevent viral attachment to cell surface receptors, and combinations thereof may be combined with the effective dosage of camptothecin.

The effective dosage is administered to the mammal one 25 or more times per day at one or more times per month for a selected period of time. The camptothecin compound is administered at approximately from 0.02 mg/kg to 10 mg/kg of body weight of the virus infected mammal per week. Alternatively the effective dosage of the camptothecin 30 compound is administered at approximately from 0.10 mg/m<sup>2</sup> to 15 mg/m<sup>2</sup> of the body surface area of the virus infected mammal per day. The effective dosage of the camptothecin compound may be administered parenterally or orally one to seven days per week.

35 A pharmaceutical formulation for treating viral diseases in mammals comprising a camptothecin compound

selected from the group consisting essentially of camptothecin, active semisynthetic camptothecin analogues, active synthetic camptothecin analogues, and combinations thereof and an inert carrier compound is also provided.

#### 5 DETAILED DESCRIPTION OF THE INVENTION

Four laboratory isolates of HIV-1 (human immunodeficiency virus) were cultured to evaluate their susceptibilities to 9-aminocamptothecin and 9-nitrocamptothecin. Two of the laboratory isolates were  
10 from naive (untreated) patients. A third laboratory isolate was resistant to Zidovudine (AZT) and the fourth isolate was resistant to AZT and Didanosine (DDI). Dimitrov, D.H., et al., *The Jour. of Inf. Dis.*, 167, 818 (1993). The following four HIV-1 strains were cultured:  
15 (1) HIV-1 strain 43.3 isolated from an adult naive (untreated) patient; (2) HIV-1 strain 114E isolated from a newborn infant naive (untreated); (3) HIV-1 strain 1048 an isolate resistant to 1  $\mu$ M zidovudine; and (4) HIV-1 strain 0885 an isolate resistant to 0.063  $\mu$ M zidovudine and 100  $\mu$ M  
20 didanosine.

Semipurified 20(S)-camptothecin lactone was purified to homogeneity as determined by analytic methods known to those skilled in the art. Derivatives 9-amino-20(S)-camptothecin lactone and 9-nitro-20(S)-camptothecin lactone  
25 were synthesized as provided by Wani, M.C., et al., *J. of Medicinal Chem.*, 29, 2358 (1986). The compounds were purified to a single peak and analyzed by high performance liquid chromatography according to known methods. The purified compounds were stored in desiccators under liquid  
30 nitrogen at -70°C. Just before use, the 9-amino-20(S)-camptothecin lactone, labile in solution or suspension, was prepared as a fine suspension in polyethylene glycol (PEG 400) (Aldrich, Milwaukee, WI) at a concentration of 1  $\mu$ g/ml. The 9-nitro-20(S)-camptothecin lactone, stable in

solution and suspension, was prepared in a similar manner and stored at -70°C.

The S form with pure stereochemistry of camptothecin and its analogues has been found to be approximately two-fold more potent than the mixtures of pure and racemic (RS) forms. The pure S preparations are preferred, however, the (RS) forms 9-amino-20(RS)-camptothecin, 9-nitro-20(RS)-camptothecin and mixtures of the analogues may be used in alternative embodiments of the invention. The compound 9-nitro-20(S)-camptothecin is converted intracellularly into 9-amino-20(S)-camptothecin and 9-nitro-20(S)-camptothecin may be used in circumstances where 9-amino-20(S)-camptothecin warrants use.

Prior to the administration of camptothecin or its analogues, it is important to purify the subject compound to homogeneity as required by the FDA regulations. The purity of the compound can be tested by high performance liquid chromatography and other appropriate methods known in the art. Also the compound should be characterized completely using IR, UV and nuclear magnetic resonance spectroscopy. It is preferred that the camptothecin compounds have a 96% or greater purity when used to practice the present invention.

The viruses were isolated by coculturing peripheral blood mononuclear cells (PBMCs) from the infected patients with phytohemagglutinin (PHA-P) (Difco, Detroit, MI) stimulated PBMCs from healthy donors. PBMCs from the HIV-1 infected patients were processed within 6 hours of collection of each virus strain according to methods known to those skilled in the art. Dimitrov, et al. (1993). The tissue culture infected dose (TCID<sub>50</sub>) of each HIV-1 strain was performed according to methods known to those skilled in the art. Dimitrov, et al. (1993).

The cultured virus isolates were assayed to determine the effects of 9-amino-20(S)-camptothecin and 9-nitro-20(S)-camptothecin on viral replication, and the

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effectiveness of the compounds on the treatment and prevention of viral diseases. Eight 24-well (8 rows of 3 wells) microculture plates (Corning Glass Works, Corning, N.Y.) were used to study the four viral assays. A separate plate was prepared to observe viral replication of each virus strain in various combinations of 9-amino-20(S)-camptothecin treated and untreated virus isolate, PBMCs, and culture medium. A separate plate was also prepared to observe viral replication of each virus strain in various combinations of 9-nitro-20(S)-camptothecin treated and untreated virus isolate, PBMCs, and culture medium.

Combinations of virus PBMCs, and culture medium were added to each row of three wells. Some or all of the components were treated with 1 nanogram/milliliter (ng/ml) of either the 9-aminocamptothecin compound or the 9-nitrocamptothecin compound. The following combinations of treated and untreated PBMCs, virus, and culture medium added to each row of three wells on a plate:

Table 1.

ROW	VIRUS	PBMC	MEDIUM
1	UNTREATED	UNTREATED	UNTREATED
2	UNTREATED	TREATED	UNTREATED
3	UNTREATED	UNTREATED	TREATED
4	TREATED	UNTREATED	UNTREATED
5	TREATED	TREATED	UNTREATED
6	TREATED	UNTREATED	TREATED
7	UNTREATED	TREATED	TREATED
8	TREATED	TREATED	TREATED

Eight million ( $8 \times 10^6$ ) phytohemagglutinin-P (PHA-P), (Difco, Detroit, MI) stimulated donor PBMCs were added to each of six 15 mm conical centrifuge tubes per plate. The cells were pelleted at  $200 \times g$  for 10 minutes at  $20^\circ - 24^\circ\text{C}$ , and the supernatant removed. Each tube received 80 TCID<sub>50</sub> of the same virus strain in 0.8 ml of Phosphate Buffer Solution (PBS), resulting in  $10 \text{ TCID}_{50}/1 \times 10^6$  PHA-P-stimulated PBMCs. After an absorption period of 1 hour at  $37^\circ\text{C}$ , the cells were pelleted again at  $200 \times g$ . The residual inoculum was removed. The cells were washed with PBS and resuspended in 8 ml of the coculture medium of PBMCs from infected patients and PHA-P stimulated PBMCs from healthy donors described above. Two (2) ml of the suspension ( $1 \times 10^6$  PBMCs/ml) was placed into each well of the plate and the plates were incubated at  $37^\circ\text{C}$  for 14 days. The camptothecin analogue was added to the virus, PBMC, and culture medium components according to the protocol of table 1 at an amount of 1 ng/ml of the component.

On day 7 of incubation, 1 ml of medium was removed from each well and replaced with 1 ml of fresh coculture

medium containing  $1 \times 10^6$  PHA-P stimulated donor PBMCs and the corresponding amount of 9-amino-20(S)-camptothecin or 9-nitro-20(S)-camptothecin where appropriate. The cultures were terminated on day 14. The supernatant was tested for HIV-1 p24 antigen concentration using methods known to those in the art. A concentration of  $\geq 30$  picogram/ml (pg/ml) was considered a positive well. Cell viability was determined by the trypan blue exclusion method.

The four strains of HIV-1 showed excellent growth in the absence of either camptothecin analogue. Treating a single component or combination of components with 1 ng/ml ( $.00275 \mu\text{M}$ ) of 9-amino-20(S)-camptothecin resulted in no detectable replication of the four HIV-1 strains being observed at 7 days or at 14 days in the culture system.

The addition of 1 ng/ml ( $.00254 \mu\text{M}$ ) of 9-nitro-20(S)-camptothecin to any single component or combination of components resulted in no detectable replication of any of the HIV-1 strains at day 7 of incubation. However, at 14 days of incubation, a slight level of viral replication was observed, indicating that equimolar or higher doses of 9-nitro-20(S)-camptothecin compared to 9-amino-20(S)-camptothecin would be required to inhibit viral replication for longer periods of time.

The susceptibility of the AZT and the AZT and DDI resistant HIV-1 viral isolates was the same as for the two isolates from naive patients. The susceptibility of the four strains of HIV-1 to 9-amino-20(S)-camptothecin and 9-nitro-20(S)-camptothecin demonstrates that susceptibility to the camptothecin analogues is independent of HIV-1 resistance to AZT and/or DDI.

At 1 ng/ml concentration, the 9-nitro-(S)-camptothecin and 9-amino-(S) camptothecin produced inhibition of HIV replication at eleven-fold lower concentration than  $0.03 \mu\text{M}$  concentration of Topotecan reported by Li, C.J., et al., Proc. Natl. Acad. of Sci., 90, 1839 (1993) and eighteen-fold lower concentration than 18.2 ng/ml or  $0.05 \mu\text{M}$  of 20-

(S)-camptothecin reported by Priol, E., et al., *Aids Res. and Human Retrov.*, 7, 65 (1991). A daily oral dose of 0.1 mg/kg 9-nitro-(S) camptothecin taken for over 5 weeks by a leukemia patient has demonstrated no discernible side effects. According to Hinz, H.R., et al., *Cancer Res.*, 54, 3096-3100 (1994) they were able to maintain 100 times the inhibiting 1 ng/ml concentration for 48 hours in a human subject with a peak concentration equaling over 1000 times the therapeutic dose and the observation of only minimal toxicity. This human subject took 1 mg/kg of 9-nitro-(S) camptothecin.

The chemical synthesis of 9-amino-20(S)-camptothecin is a low yield, costly procedure. Moreover, 9-amino-20(S)-camptothecin is light sensitive, heat sensitive and oxygen sensitive. The compound is unstable and produces decomposition products that are toxic when administered to nude mice, whereas the toxicity of 9-nitro-20(S)-camptothecin is considerably less.

Although the 1 ng/ml (.00254  $\mu$ M) of the 9-nitro-20(S)-camptothecin treatment demonstrated less inhibition of HIV-1 replication at fourteen days than was observed with equal amounts, but slightly lower than equimolar doses of 9-amino-20(S)-camptothecin, because 9-nitro-20(S)-camptothecin is highly stable both in crystalline form and in suspension, is less costly to produce, and effective plasma concentrations of 9-amino-20(S)-camptothecin are obtainable by oral administration of 9-nitro-20(S)-camptothecin, 9-nitro-20(S)-camptothecin has many advantages in clinical use. Accordingly, 9-nitro-20(S)-camptothecin can be administered in all diseases where 9-amino-20(S)-camptothecin is effective alone or in combination with other medications.

The greater potency of viral inhibition without increased toxicity allows the use of a lower dose of oral 9-nitro-20(S)-camptothecin with a further decrease in toxicity and side effects. A simple oral dose of 0.1 mg/kg

9-nitro-20(S)-campt thecin in a human v lunt er r ached a plasma level f 483 ng/ml at 3.4 hours. With conversion to 9-amino-20(S)-camptothecin, a plasma level of 14 ng/ml was reached at 10.3 hours. Such plasma levels markedly exceed  
5 the ng/ml concentration needed to inhibit HIV-1 replication.

Diseases which may be treated by camptothecin alone or in combination with other medications include, for example, retroviral diseases, such as, HIV-1, HIV-2, human T-cell  
10 leukemia virus-I (HTLV-I), HTLV-II, HTLV-III, simian immunodeficiency virus (SIV), lymphadenopathy-associated virus (LAV-2), simian T-lymphotrophic virus-I (STLV-I),  
STLV-II, STLV-III, simian B-lymphotrophic (SBL) virus, Gibbon ape leukemia virus (GALV), bovine leukemia virus  
15 (BLV), equine infectious anemia virus (EIAV), feline leukemia virus (FELV), murine leukemia virus (MuLV), avian leukosis virus (ALV); other virus infections such as hepadnaviridae (Hepatitis B); herpesviridae (Herpes simplex I, Herpes simplex II, Varicella-Zoster, Epstein-Barr virus  
20 and cytomegalovirus); parvoviridae (human parvovirus B-19); papovaviridae (human papilloma virus types 1 to 60, JC and BK viruses); pox viruses (variola major, variola minor, vaccinia, monkey pox, cowpox, paravaccinia or milker's nod  
virus, parapox or ORF virus, molluscum contagiosum) and  
25 cancers, lymphomas and other leukemias. Generally, such diseases will be treated with camptothecin compounds when the symptoms attributable to the particular viral infection are diagnosed. The camptothecin compounds, however, may also be used to prevent the manifestation of the symptoms  
30 of viral diseases following an exposure to a particular virus.

In alternate embodiments of the invention, immunomodulator drugs, certain cytokine agonists and certain cytokine inhibitors, as well as drugs which prevent  
35 or inhibit retroviral adhesion to cell surface receptors and penetration into the cell may also be used in



combination with the camptothecin analogue to treat or prevent viral infections. Hepadnavirus replication-inhibiting compounds such as interferon alpha-2 (for the treatment of acute and chronic Hepatitis B), herpesvirus replication-inhibiting compounds such as acyclovir, ganciclovir, and foscarnet, and papovavirus replication-inhibiting compounds, such as interferon alpha-n3, may also be used in combination with the camptothecin analogue or combinations of analogues to treat or prevent viral infections. The means of exposure to a particular viral disease does not affect the efficacy of the camptothecin compounds in inhibiting viral replication.

In other embodiments of the invention, mixtures of 20-(S)-camptothecin, 9-nitro-20(S)-camptothecin, 20(S)-camptothecin or any other viral replication-inhibitory natural, semisynthetic, or synthetic analogue of a compound or medication which inhibits the long terminal repeat of retroviruses, such as B lapacone and curcumin, can be used to enhance effectiveness of antiretroviral treatment by reducing the dose required, adverse reactions to treatment, and delaying onset of drug resistance by the virus. Natural, semisynthetic or synthetic analogues of the B lapacone and curcumin compounds may also be used to enhance the effectiveness of antiviral treatments. In yet another embodiment of the present invention, an effective antiretroviral treatment may, in addition to the camptothecin and other long terminal repeat inhibitors, include reverse transcriptase inhibitors, TAT inhibitors and protease inhibitors, rev protein inhibitors, and rev responsive element (rre) inhibitors.

As used herein, an effective dosage of virus replication inhibiting camptothecin and/or its analogues is intended to mean a dosage of the compound that will inhibit replication or transcription of the virus and cause regression and palliation of the viral disease or eliminate the disease entirely.

An effective dose comprises approximately 0.02 mg/kg to 10 mg/kg of body weight of the mammal per week. The effective dosage can be administered in daily treatments or subdivided into a regimen of fewer treatments such as two administrations per week. For example, 9-nitro-20(S)-camptothecin orally administered at 0.1 mg/kg of body weight per day in humans produces a therapeutic blood level with non-discernable or minimal side effects. A single oral dose of 1 mg/kg produces a blood concentration one hundred fold greater than the concentration required to inhibit viral activity in humans with minimal side effects.

Alternatively, an effective dosage of the camptothecin compounds in the present invention can range from approximately 0.10 mg/m<sup>2</sup> of body surface per day to approximately 15.0 mg/m<sup>2</sup> of body surface per day in humans. Higher doses may be used, however, patients will need to be monitored for signs of toxicity. Effective dosages for animals will also range from approximately 0.10 mg/m<sup>2</sup> to approximately 15.0 mg/m<sup>2</sup>. The interrelationship of dosages for animals of various sizes and species, and for humans, based on milligram/meter<sup>2</sup> (mg/m<sup>2</sup>) of body surface is described by Freireich, E.J., et al., *Cancer Chemother Rp.*, 50 (4):219 (1966). Body surface area may be determined approximately from the height and weight of an individual. Scientific Tables, Geigy Pharmaceuticals, Ardley, N.Y., pp. 537-538 (1970).

The camptothecin analogues may be administered parenterally, including subcutaneously, intraperitoneally, intramuscularly, and intravenously. The camptothecin analogues may be administered orally in suitable oral dosage forms to be described below. The plasma concentration of 9-amino-20(S)-camptothecin in patients safely administered 9-nitro-20(S)-camptothecin orally or 9-amino-20(S)-camptothecin in intravenous formulation are many fold greater than the concentration of 9-nitro-20(S)-camptothecin or 9-amino-20(S)-camptothecin need d to

inhibit HIV-1 replication in tissue culture. Transdermal preparations, electrical patch, electroporation, and sensor rate regulated electrical patch may be administered in a pharmaceutical formulation.

5       An effective dosage of the camptothecin analogues may be administered as pharmaceutical formulations including acceptable carriers or diluents, such as Tween/NaCl (for example, Tween 80:0.15 NaCl), Cremaphor EL (D-12, NIH),  
10       lipophilic compounds and water-based solvents, such as normal saline or phosphate buffered saline solutions for water soluble analogues.

      The pharmaceutical formulations may be administered parenterally, including subcutaneously, intraperitoneally,  
15       intramuscularly, and intravenously as described above. The formulation may be administered orally, intranasally, or by transdermal patch in pharmaceutical preparations by well known means. The lipophilic state compounds, 20(S)-camptothecin and 9-nitro-20(S)-camptothecin, may be  
20       administered as pure crystals in gelatin capsules on an empty stomach followed by an acid drink such as orange juice.

      Capsules containing camptothecin or camptothecin analogues may comprise any well known pharmaceutically  
25       acceptable material, such as gelatin or cellulose derivatives, as carriers. Tablets or capsules may contain the camptothecin compound combined with an acceptable pharmaceutical excipient. Tablets may be formulated in accordance with conventional procedures employing solid  
30       carriers and/or lubricants known in the art. Examples of solid carriers are starch, sugar, and bentonite. The camptothecin compound may be dried and administered in the form of a hard shell tablet or capsule containing lactose or mannitol as a binder, and conventional fillers and  
35       tableting agents. The camptothecin compound should be

present at from approximately 1 to 20 mg of the tablet or capsule.

The unstable 9-amino-20(S)-camptothecin compound can be formulated in suitable oral dosage forms by preparing the lyophilized drug in an ampoule and having the emulsifier in a separate vial such that the two components are formulated before administration of the effective dosage. Parenteral preparations of the lipophilic stable and unstable camptothecin compounds may be prepared as lyophilized crystals in an ampoule with sterile solvents comprising, for example, polyethylene glycol USP, ethylene glycol USP, ethyl alcohol USP, purified water USP, and mixtures thereof. The stable compounds can be stored in sterile solutions for parenteral use or in liquid form in suitable capsules or bottles for oral use. The quantity of effective dose supplied by each capsule will depend upon the total dosage to be reached.

The pharmaceutical formulation for treating viral diseases in mammals may also be administered as a sterile ophthalmic suspension and as an ointment for local application to the conjunctiva of the eye or as a slow release lozenge.

The scheduling and duration of treatment may vary with the patient's response and the use of concomitant medication with potential separate or cumulative toxicities. In patients with AIDS, camptothecin compounds may be required on a daily to twice weekly schedule indefinitely. One or more capsules or other form of administration may be taken one or more times per day, for example, the total daily dose may be divided into equal halves given at approximately twelve hour intervals. In some situations, an effective dosage may be taken one or more times per day at scheduled times during a weekly or monthly administration schedule.

The description of the process and embodiment is illustrative of the invention and is not intended to place

any limitation on the claims of invention. Those skilled in the art will recognize the various modes of practicing the invention described herein.

What is claimed is:

1           1. A method of inhibiting viral replication  
2 comprising the steps of:

3           (a) preparing an effective dosage of a camptothecin  
4 compound selected from the group consisting essentially of  
5 camptothecin, active semisynthetic camptothecin analogues,  
6 active synthetic camptothecin analogues, and combinations  
7 thereof; and

8           (b) introducing the effective dosage to a virus.

1           2. The method of inhibiting viral replication of  
2 claim 1, wherein the virus may be selected from the group  
3 of viruses consisting essentially of retroviruses, DNA  
4 viruses, DNA-mediated RNA-containing viruses, and viruses  
5 containing viral topoisomerase I.

1           3. The method of inhibiting viral replication of  
2 claim 1, wherein the effective dosage comprises an  
3 effective amount of a camptothecin compound selected from  
4 the group consisting of 9-amino-20(S)-camptothecin, 9-  
5 amino-20(RS)-camptothecin, 9-nitro-20(S)-camptothecin, 9-  
6 nitro-20(RS)-camptothecin, 20-(S)-camptothecin, and 20-  
7 (RS)-camptothecin, and combinations thereof.

1           4. The method of inhibiting viral replication of  
2 claim 1, wherein step (a) further comprises adding an  
3 antiretroviral compound selected from the group consisting  
4 of HIV long terminal repeat inhibiting compounds, reverse  
5 transcriptase inhibitors, protease inhibitors, TAT  
6 inhibitors, rev protein inhibitors, and rre inhibitors and  
7 combinations thereof to the effective dosage of  
8 camptothecin compound.

1           5.    The method of inhibiting viral replicati n of  
2   claim 1, wh r in st p (a) further c mpris s adding on or  
3   more antiretroviral compounds selected from the group

4   consisting of B lapacone, B lapacone analogues, curcumin  
5   analogues, zidovudine, didanosine, rev protein inhibitors,  
6   rre inhibitors, interferon alpha-2b, interferon alpha-n3,  
7   acyclovir, gangcylcovir, foscarnet, and combinations  
8   thereof to the effective dosage of camptothecin compound.

1           6.    The method of inhibiting viral replication of  
2   claim 1, wherein step (a) further comprises adding  
3   immunoadjuvant drugs, cytokine agonists, cytokine  
4   inhibitors, and combinations thereof to the effective  
5   dosage of camptothecin compound.

1           7.    The method of inhibiting viral replication of  
2   claim 1, wherein the effective dosage is introduced to the  
3   virus one or more times per day on one or more days per  
4   month for a selected period of time.

1           8.    A method of treating viral diseases in mammals  
2   comprising the steps of:

3           (a)   preparing an effective dosage of a camptothecin  
4   compound selected from the group consisting essentially of  
5   camptothecin, active semisynthetic camptothecin analogues,  
6   active synthetic camptothecin analogues, and combinations  
7   thereof; and

8           (b)   administering the effective dosage to a virus  
9   infected mammal.

1           9.    The method of treating viral diseases in mammals  
2   of claim 8, wherein the effective dosage of the  
3   camptothecin compound is administered to prevent the onset  
4   of viral disease in the virus infected mammal.

1           10. The method of treating viral diseases in mammals  
2 of claim 8, wherein the camptothecin compound is 9-nitro-  
3 20(S)-camptothecin.

1           11. The method of treating viral diseases in mammals  
2 of claim 8, wherein the camptothecin compound is 9-amino-  
3 20(S)-camptothecin.

1           12. The method of treating viral diseases in mammals  
2 of claim 8, wherein the camptothecin compound is 20-(S)-  
3 camptothecin.

1           13. The method of treating viral diseases in mammals  
2 of claim 8, wherein the camptothecin compound is 9-nitro-  
3 20(RS)-camptothecin.

1           14. The method of treating viral diseases in mammals  
2 of claim 8, wherein the camptothecin compound is 9-amino-  
3 20(RS)-camptothecin.

1           15. The method of treating viral diseases in mammals  
2 of claim 8, wherein the camptothecin compound is 20-(RS)-  
3 camptothecin.

1           16. The method of treating viral diseases in mammals  
2 of claim 8, wherein step (a) further comprises adding on  
3 or more antiretroviral compounds selected from the group  
4 consisting of HIV long terminal repeat inhibiting  
5 compounds, reverse transcriptase inhibitors, protease  
6 inhibitors, TAT inhibitors, rev protein inhibitors, and rr  
7 inhibitors and combinations thereof to the effective dosage  
8 of camptothecin compound.

1           17. The method of treating viral diseases in mammals  
2 of claim 8, wherein step (a) further comprises adding an  
3 antiretroviral compound selected from the group consisting



4 f B lapacone, curcumin, curcumin analogues, zid vudine,  
5 didan sin , rev prot in inhibitors, rre inhibit rs,  
6 immunoadjuvant drugs, c rtain cytokine ag nists, cytokin  
7 inhibitors, interferon alpha-2b, interferon alpha-n3,  
8 acyclovir, gangcylcovir, foscarnet, and combinations thereof  
9 to the effective dosage of camptothecin compound.

1 18. The method of treating viral diseases in mammals  
2 of claim 8, wherein step (a) further comprises adding  
3 immunoadjuvant drugs, cytokine agonists, cytokine  
4 inhibitors, and combinations thereof to the effective  
5 dosage of camptothecin compound.

1 19. The method of treating viral diseases in mammals  
2 of claim 8, wherein the effective dosage is administered to  
3 the mammal one or more times per day on one or more days  
4 per month for a selected period of time.

1 20. The method of treating viral diseases in mammals  
2 of claim 8, wherein the effective dosage of the  
3 camptothecin compound is approximately from 0.02 mg/kg to  
4 10 mg/kg of body weight of the virus infected mammal per  
5 week.

1 21. The method of treating viral diseases in mammals  
2 of claim 8, wherein the effective dosage of the  
3 camptothecin compound is approximately from 0.10 mg/m<sup>2</sup> to  
4 15 mg/m<sup>2</sup> of the body surface area of the virus infected  
5 mammal per day.

1 22. The method of treating viral diseases in mammals  
2 of claim 8, wherein the effective dosage of the  
3 camptothecin compound is administered parenterally.

1           23. The method of treating viral diseases in mammals  
2 of claim 8, wherein the effective dosage of the  
3 camptothecin compound is administered orally.

1           24. A pharmaceutical formulation for treating viral  
2 diseases in mammals comprising:

3           (a) a camptothecin compound selected from the group  
4 consisting essentially of camptothecin, active  
5 semisynthetic camptothecin analogues, active synthetic  
6 camptothecin analogues, and combinations thereof; and

7           (b) an inert carrier compound.

1           25. The pharmaceutical formulation for treating viral  
2 diseases in mammals of claim 24, wherein the formulation is  
3 selected from the group consisting of sterile ophthalmic  
4 suspensions, sterile ophthalmic ointments for local  
5 application to the conjunctiva of the eye, tablets,  
6 capsules, and slow release lozenges.

1           26. The pharmaceutical formulation for treating viral  
2 diseases in mammals of claim 24, wherein the camptothecin  
3 compound is present at from approximately 1 to 20 mg of the  
4 total formulation.

1           27. The pharmaceutical formulation for treating viral  
2 diseases in mammals of claim 24, wherein the camptothecin  
3 compound is 9-nitro-20(S)-camptothecin.

1           28. The pharmaceutical formulation for treating viral  
2 diseases in mammals of claim 24, wherein the camptothecin  
3 compound is 9-amino-20(S)-camptothecin.

1           29. The pharmaceutical formulation for treating viral  
2 diseases in mammals of claim 24, wherein the camptothecin  
3 compound is 20-(S)-camptothecin.

1           30. Th pharmaceutical formulation for tr ating viral  
2   diseas s in mammals of claim 24, wher in the camptothecin  
3   compound is 9-nitro-20(RS)-camptothecin.

1           31. The pharmaceutical formulation for treating viral  
2   diseases in mammals of claim 24, wherein the camptothecin  
3   compound is 9-amino-20(RS)-camptothecin.

1           32. The pharmaceutical formulation for treating viral  
2   diseases in mammals of claim 24, wherein the camptothecin  
3   compound is 20-(RS)-camptothecin.

1           33. The pharmaceutical formulation for treating viral  
2   diseases in mammals of claim 24, wherein the inert carrier  
3   is selected from the group of compounds consisting of  
4   gelatin, cellulose, cellulose derivatives, pharmaceutical  
5   excipients, pharmaceutical lubricants, starch, sugar,  
6   lactose, mannitol, polyethylene glycol USP, ethylene glycol  
7   USP, ethyl alcohol USP, purified water USP, and mixtures  
8   thereof.

1           34. The pharmaceutical formulation for treating viral  
2   diseases in mammals of claim 24, wherein the formulation is  
3   administered in a parenteral dosage form.

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: <b>PCT/US95/12250</b> (22) International Filing Date: 26 September 1995 (26.09.95) (30) Priority Data: 08/319,451 6 October 1994 (06.10.94) US (71)(72) Applicant and Inventor: <b>ATLAS, Leon, T. [US/US]; 5034 Heatherglen, Houston, TX 77096 (US).</b> (74) Agents: <b>VADEN, Frank, S., III et al.; Vaden, Eickenroht, Thompson &amp; Feather, L.L.P., Suite 1100, One Riverway, Houston, TX 77056-1982 (US).</b>	(81) Designated States: <b>AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).</b>  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  (88) Date of publication of the international search report: 30 May 1996 (30.05.96)	
(54) Title: <b>USE OF CAMPTOTHECIN OR DERIVATIVES THEREOF FOR THE MANUFACTURE OF A MEDICAMENT FOR THE TREATMENT OF VIRAL DISEASES</b>  (57) Abstract  The present invention provides methods of inhibiting viral replication by preparing an effective dosage of a camptothecin compound and introducing the effective dosage to the virus. Retrovirus, DNA virus, and topoisomerase I containing virus replication is inhibited. The camptothecin compound may be 9-amino-20(S)-camptothecin, 9-nitro-20(S)-camptothecin, 20-(S)-camptothecin, or any other active camptothecin compound, active semisynthetic camptothecin analogue, or active synthetic camptothecin analogue. One or more antiviral drugs may be combined with one or more of the aforementioned camptothecin compounds. A method of treating viral diseases in mammals comprising preparing an effective dosage of a camptothecin compound selected from the group consisting essentially of camptothecin, active semisynthetic camptothecin analogues, active synthetic camptothecin analogues, and combinations thereof. The dosage is administered to the infected mammal. Pharmaceutical formulations are also provided.		

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# INTERNATIONAL SEARCH REPORT

Int. Appl. No.  
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A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K31/435

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	EP,A,0 685 481 (INDENA SPA) 6 December 1995 see page 3, line 26 - line 40; claims 1,7,8	1,2,8,9, 24
X	WO,A,91 16904 (THE UNITED STATES OF AMERICA) 14 November 1991 see the whole document	1-34
X	US,A,4 894 456 (MONROE E. WALL ET AL.) 16 January 1990  see column 3, line 60 - column 5, line 7 see abstract see column 9, line 40 - column 11, line 28	24-26, 30,31, 33,34
	-/--	

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A,5 321 140 (DANIEL L. COMINS ET AL.) 14 June 1994 see column 6, line 44 - column 7, line 12 ---	3-7,9-34
X	J.BIOL.CHEM, vol. 269, no. 10, 11 March 1994 pages 7051-7054, CHIANG J. LI ET AL. 'Camptothecin inhibits tat-mediated transactivation of type 1 human immunodeficiency virus' see abstract see page 7054, left column, paragraph 2	1,2,8
Y	---	3-7,9-34
X	INT.CONF.AIDS, vol. 10, no. 1, 7 August 1994 - 12 August 1994 page 102 HIDEHIRO TAKAHASHI ET AL. 'Inhibitory mechanism of camptothecin on HIV-1 replication' see abstract no. PA0028	1,2,8
Y	---	3-7,9-34
X	ARCH. VIROL., vol. 110, no. 1-2, 1990 pages 121-127, Y.YAMADA 'Role of DNA topoisomerase I in the replication of herpes simplex virus type 2' see the summary	1,2,8
Y	-----	3-7,9-34

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/12250

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-685481	06-12-95	CA-A- 2127763 NO-A- 942633	01-12-95 01-12-95
WO-A-9116904	14-11-91	US-A- 5422344 AU-B- 7880791	06-06-95 27-11-91
US-A-4894456	16-01-90	US-A- 4981968 US-A- 5401747 US-A- 5106742 US-A- 5244903 US-A- 5227380 US-A- 5122526 US-A- 5364858	01-01-91 28-03-95 21-04-92 14-09-93 13-07-93 16-06-92 15-11-94
US-A-5321140	14-06-94	US-A- 5162532 US-A- 5200524 CA-A- 2112351 EP-A- 0565621 JP-T- 6504062 US-A- 5254690 US-A- 5258516 US-A- 5264579 US-A- 5315007 WO-A- 9211263 US-A- 5212317 US-A- 5191082 US-A- 5247089 US-A- 5243050	10-11-92 06-04-93 09-07-92 20-10-93 12-05-94 19-10-93 02-11-93 23-11-93 24-05-94 09-07-92 18-05-93 02-03-93 21-09-93 07-09-93